



VICTORIA UNIVERSITY
MELBOURNE AUSTRALIA

Maximal exercise increases mucosal associated invariant T cell frequency and number in healthy young men

This is the Accepted version of the following publication

Hanson, Erik, Danson, E, Nguyen-Robertson, CV, Fyfe, JJ, Stepto, Nigel, Bartlett, DB and Sakkal, Samy (2017) Maximal exercise increases mucosal associated invariant T cell frequency and number in healthy young men. *European Journal of Applied Physiology*, 117 (11). 2159 - 2169. ISSN 1439-6319

The publisher's official version can be found at
<https://link.springer.com/article/10.1007/s00421-017-3704-z>
Note that access to this version may require subscription.

Downloaded from VU Research Repository <https://vuir.vu.edu.au/36367/>

MAXIMAL EXERCISE INCREASES MUCOSAL ASSOCIATED INVARIANT T CELL
FREQUENCY AND NUMBER IN HEALTHY YOUNG MEN

Erik D. Hanson^{1,2,3}, E. Danson¹, Catriona V. Nguyen Robertson³, Jackson J. Fyfe^{2,4}, Nigel K.
Stepito^{2,5,6}, David B. Bartlett⁷ and Samy Sakkal^{2,3}

¹Department of Exercise & Sports Science, University of North Carolina, Chapel Hill, NC, USA

²Institute of Sport, Exercise, and Active Living, Victoria University, Melbourne, VIC, 8001
Australia

³Centre for Chronic Disease, College of Health and Biomedicine, Victoria University,
Melbourne, VIC, 8001 Australia

⁴School of Exercise and Nutrition Sciences, Deakin University, Burwood, VIC, 3125 Australia

⁵Monash Centre for Health Research and Implementation, School of Public Health and
Preventative Medicine, Monash University, Clayton VIC, 3800, Australia

⁶Australian Institute for Musculoskeletal Science, Victoria University, St. Albans, VIC, 3021,
Australia

⁷Division of Medical Oncology, Department of Medicine, Duke University, Durham, NC, USA

Running Title: MAIT Cells and Acute Exercise

Corresponding Author:

Erik D. Hanson, PhD

Department of Exercise and Sport Science

CB#8605, 315 Woollen Gym

University of North Carolina at Chapel Hill

Chapel Hill, NC 27599

Phone: 919.962.0816

Email: edhanson@email.unc.edu

Key Words: TCR V α 7.2, exercise immunology, MAIT cells

ABBREVIATIONS

7-AAD = 7-amino-actinomycin D

CTL = cytotoxic T lymphocyte

GXT = graded exercise test

HbA1C = glycated hemoglobin

MAIT cells = mucosal associated invariant T cell

PBMC = peripheral blood mononuclear cell

TCR = T cell receptor

VO₂max = maximal oxygen uptake

W = watts

ABSTRACT

Mucosal associated invariant T (MAIT) cells have properties of both the innate and acquired immune systems. While the response to vigorous exercise has been established for most leukocytes, MAIT cells have yet to be investigated. **PURPOSE:** To determine if MAIT cell lymphocytosis occurs with acute maximal aerobic exercise and if this response is influenced by exercise duration, cardiovascular fitness, or body composition. **METHODS:** Twenty healthy young males with moderate fitness levels performed an extended graded exercise test until volitional fatigue. Peripheral blood mononuclear cells were isolated from venous blood obtained prior and immediately after exercise and were labelled to identify specific T cell populations using flow cytometry. **RESULTS:** The percentage of MAIT cells relative to total T cells significantly increased from 3.0 to 3.8% and absolute MAIT cell counts increased by 2.2 fold following maximal exercise. MAIT cell subpopulation proportions were unchanged with exercise. Within cytotoxic T lymphocytes (CTL), MAIT cells consisted of 8% of these cells and this remained constant after exercise. MAIT cell counts and changes with exercise were not affected by body composition, $\text{VO}_{2\text{peak}}$, or exercise duration. **CONCLUSIONS:** Maximal exercise doubled MAIT cell numbers and showed preferential mobilization within total T cells but the response was not influenced by fitness levels, exercise duration, or body composition. These results suggest that acute exercise could be used to offset MAIT cell deficiencies observed with certain pathologies. MAIT cells also make up a substantial proportion of CTLs, which may have implications for cytotoxicity assays using these cells.

INTRODUCTION

Mucosal associated invariant T (MAIT) cells are pro-inflammatory cells that occupy a unique niche within human immunity by displaying characteristics of both the innate and acquired immune systems (Godfrey et al. 2015). Identified by expression of the semi-invariant T cell receptor (TCR) V α 7.2 (or via MR1 loaded tetramers), these innate-like T cells develop in the thymus after major histocompatibility complex class I-related protein (MR1)-expressing hematopoietic cells are selected. These hematopoietic cells in turn select for the MAIT cells, and while these cells have been identified as MR1⁺ double positive thymocytes in mice, there is some ambiguity as to whether these cells are also responsible for selecting human MAIT cells (Gold et al. 2014; Gold et al. 2015; Koay et al. 2016; Tilloy et al. 1999). MAIT cell residing in the thymus and in cord blood display naïve phenotypes, with CD45RO expression being acquired later (Dusseaux et al. 2011; Gold et al. 2013). Final development occurs extrathymically and is dependent on microbial colonization (Koay et al. 2016) and high levels of CD161 expression (Billerbeck et al. 2010; Martin et al. 2009a; Takahashi et al. 2006), with MAIT cells largely being either CD8⁺ or CD4⁺CD8⁻ (Godfrey et al. 2015).

Upon thymic egress, the range of circulating MAIT cells in healthy individuals varies between 2-5% of CD3⁺ cells (Godfrey et al. 2015; Magalhaes et al. 2015; Serriari et al. 2014) and are directed toward mucosal tissue including the liver, gastrointestinal tract, spleen, and lungs (Dusseaux et al. 2011). At these sites, MAIT cells interact with commensal flora and B-cells, initiating MAIT TCR adaptations, proliferation, maturation/activation (Gold et al. 2015) and ultimately accumulation within mucosal tissues (Martin et al. 2009a; Treiner et al. 2003). However, it remains unknown if MAIT cells after migrating to mucosal tissue re-enter circulation. In the absence of rigorous experimental data (such as intrathymic injections), it is not

possible to be certain that all circulating MAIT cells are antigen-naïve and this may account for both the heterogeneity in MAIT cell phenotype and isolating frequencies (Gherardin et al. 2016).

MAIT cells were previously associated exclusively with antibacterial responses (Dusseaux et al. 2011; Gold et al. 2010) following exposure to riboflavin metabolites (Chen et al. 2017), leading to expression of the pro-inflammatory cytokines IFN- γ , TNF α , and IL-17 (Chen et al. 2017; Ussher et al. 2014). However, recent evidence suggests a possible role for MAIT cells in response to viral infection. MAIT cell numbers are reduced during HIV (Cosgrove et al. 2013; Fernandez et al. 2015) and fatal influenza A infections (Loh et al. 2016) and show increased activation markers and intracellular IFN γ and granzyme B levels following co-culture with influenza infected cells (Loh et al. 2016). This activation potentially primes MAIT cells for enhanced effector function against secondary bacterial infections that accompany lung viruses as a means of enhanced immunity.

Several pathological conditions are associated with altered MAIT cell number and function. Irritable bowel syndrome and Crohn's disease present with reduced frequency of systemic MAIT cells and higher IL-17 secretion whereas MAIT cell numbers within the intestinal mucosa show conflicting results (Hiejima et al. 2015; Serriari et al. 2014). Obesity and diabetes were also shown to reduce peripheral MAIT cell numbers with a subsequent increase within adipose tissue and higher IL-17 production (Carolan et al. 2015; Magalhaes et al. 2015). Individuals with severe asthma have been reported to have reduced MAIT cell counts in both the blood and lung tissue (Hinks et al. 2015) with the magnitude of the decrement being correlated with clinical severity, indicating a potential relationship between MAIT cells and respiratory health.

Therapies that mobilize MAIT cells or cause tissue accumulation may reduce symptoms from disorders associated with reduced mucosal immunity. Acute aerobic exercise induces robust increases in circulating immune cell numbers and to a lesser extent, cell function. Multi-fold transient increases in natural killer cells, cytotoxic T lymphocytes, naïve T-helper cells, eosinophils, granulocytes and monocytes are routinely observed following acute exercise, with the magnitude of response dependent on the duration and intensity of the bout (Bishop et al. 2014; Gabriel et al. 1992; Nieman et al. 1992). Specifically, CD3⁺ T cells along with the CD4⁺ and CD8⁺ subpopulations exhibit a classical transient biphasic response, where lymphocytosis is observed immediately after exercise and subsequent lymphocyte egress develops during recovery (Gleeson and Bishop 2005; Hansen et al. 1991; Simpson et al. 2008). However, the effect of acute exercise on circulating MAIT cells frequency has yet to be assessed. While moderate levels of exercise training have been associated with lower rates of respiratory tract infection (Martin et al. 2009b; Nieman 1994), it is also unknown if MAIT cells contribute to the improvements in immunity seen with other cell types during and after exercise training (Walsh et al. 2011). A robust increase in MAIT cell with acute exercise in healthy individuals would provide a rationale to explore the use of exercise-induced lymphocytosis to enhance circulating MAIT cell counts during deficiencies. As obesity (Carolan et al. 2015; Magalhaes et al. 2015), fitness levels (Hong et al. 2005), and exercise intensity and duration (Gleeson and Bishop 2005) influence circulating lymphocyte numbers, these factors need to be considered as possible mediators of the MAIT cell response to exercise.

CD8⁺ T lymphocytes have traditionally been defined by both CD3 and CD8. Previous exercise studies that failed to include both markers may inadvertently include natural killer cells (Campbell et al. 2008), leading to inaccurate proportions within the CD8⁺ cells. Moreover, there

are at least two distinct populations of CD8⁺ T lymphocytes: cytotoxic T lymphocytes (CTL) that respond to viral peptide presentation (Zhang and Bevan 2011) and MAIT cells that respond predominately to bacteria (Chen et al. 2017; Dusseaux et al. 2011; Gold et al. 2010). It has been shown that ~10% of CD8⁺ lymphocytes have now been identified as MAIT cells by virtue of V α 7.2 and CD161^{hi} expression (Cosgrove et al. 2013; Dusseaux et al. 2011; Fergusson et al. 2016; Walker et al. 2012). This calls into question previous work that has failed to exclude V α 7.2⁺CD161⁺ cells from their CTL analysis.

As such, the purpose of this study is to determine if MAIT cell lymphocytosis occurs with acute maximal aerobic exercise and if this response is influenced by exercise duration, cardiovascular fitness, or body composition (% body fat). A secondary purpose of the study is to investigate the percentage of CTLs that are MAIT cells and to ascertain if these proportions change following acute exercise. We hypothesized that MAIT cell counts will significantly increase following maximal aerobic exercise and the proportion of MAIT cells (relative to CD3⁺ T cells and CD8⁺ T cell population) will both increase with exercise. Moreover, we anticipate that lower fitness levels (and shorter exercise time) and higher body fat will be associated with reduced MAIT cells counts.

METHODOLOGY

Participants

20 healthy young males [mean age 28 (5 y), height 180.6 (6.4 cm), mass 81.1 (14.1 kg); Table 1] volunteered to participate in this study. Participants were recreationally active in that they engaged in physical activity at least twice per week for >30 min and were free from cardiorespiratory abnormalities and lower extremity injuries. Written informed consent was obtained from participants prior to starting the trial and all procedures were approved by Victoria University Human Research Ethic Committee.

Diet and Exercise Control and Body Composition

For 24 h prior to all testing, participants refrained from structured exercise and recorded a detailed food diary. Participants reported to the laboratory after an overnight fast and body composition was determined using dual-energy x-ray absorptiometry (Discovery W, Hologic Inc., Bedford, MA, USA). Fat free mass was determined as Total mass – Fat mass – Bone mineral content, as recommended by the manufacturer. The scanner was calibrated daily and the same certified densitometry technician completed and analyzed all scans.

Graded Exercise Test (GXT) and Blood Sampling

Prior to the GXT, a venous catheter was inserted into an antecubital forearm vein for repeat blood sampling and a resting blood sample was obtained. The GXT was comprised of 4 min stages with 30 sec passive recover at the end of each stage. Participants were required to maintain a peddling cadence of ~70 rpm during each 4 min stage. Initial resistance on the electromagnetically-braked cycle ergometer (Lode, Groningen, The Netherlands) was set at 60, 90, or 120 Watts (W) as determined during familiarization (see below) to ensure a maximum of 10 stages. Each subsequent stage increased by 30 W until volitional exhaustion, defined as an

inability to maintain a cadence greater than 60 rpm. Immediately following the GXT, a second blood sample was obtained.

Following the blood sampling, a 5 min active recovery was initiated at 20 W, after which participants again cycled to exhaustion at a workload corresponding to 105% of W_{peak} achieved during the GXT, as described previously (Rossiter et al. 2006). Expired gases were sampled every 15 sec using automated gas analysers (Moxus Modular VO_2 System, AEI Technologies, Pittsburgh, PA, USA) and peak oxygen uptake (VO_{2peak}) was determined based on the two highest consecutive 15 s values achieved during the test (Fyfe et al. 2016). Gas analyzers were calibrated prior to each test using known gas concentrations (21.0% O_2 and 0.03% CO_2 , 16.0% O_2 and 4.0% CO_2).

Familiarization

Subjects completed a familiarization session 2 to 6 days prior to the GXT to determine initial workload for the GXT. The familiarization trial involved completion of the GXT protocol at an initial workload of 60 W. Participants completed as many 4 min stages with 30 sec passive recovery as possible until volitional exhaustion (defined as inability to maintain a cadence greater than 60 rpm). The number of stages completed was noted, and the starting resistance for the GXT was selected to ensure completion of between 6 and 10 stages.

Hematology Analysis

Complete blood counts were determined using whole blood samples obtained before and after the GXT (Sysmex KX-21N, Kobe, Japan). All samples were analyzed in duplicate with a maximal white blood cell difference of 0.1 cells/ μ L and the values were averaged. Plasma volume shifts with exercise were determined as described previously (Dill and Costill 1974).

Peripheral Blood Mononuclear Cells (PBMC) Isolation and Immunofluorescence Labeling

Whole blood was diluted in a 1:1 ratio with phosphate buffered saline and PBMCs were isolated using density gradient centrifugation and SepMate tubes following manufacturer instructions (StemCell Technologies Vancouver, British Columbia, Canada). PBMCs were washed, counted via hemocytometer, and 2×10^6 cells were aliquoted for immunofluorescence labeling.

Cell phenotyping was determined by direct immunofluorescence labeling of cell surface proteins with mouse anti-human monoclonal antibodies [PE TCR V α 7.2; PE/Cy7 CD8; APC/Cy7 CD3; PerCp/Cy5.5 CD45; Brilliant Violet CD161 (Biolegend, San Diego, CA, USA); V500 CD4 (BD Biosciences, San Jose, CA, USA)] in 100 μ L of cell staining buffer (Biolegend, San Diego, CA, USA) for 15 min at 4°C in the dark. Excess antibody was washed and the cells were resuspended in 200 μ L of cell staining buffer prior to flow cytometry.

Flow Cytometry

Cells were analyzed via flow cytometry on a BD FACSCanto II (BD Bioscience, CA, USA) running on FACSDIVA v6.1 (BD Bioscience, CA, USA) software. Single cells were determined using forward light-scattering properties for width and height. CD45⁺ was used to distinguish white blood cells and lymphocytes were identified and gated using the forward and side light-scattering properties. To determine total MAIT cell number and subpopulations, V α 7.2⁺CD161⁺ cells were identified as MAIT cells from the CD3⁺ population with gating on CD8⁺ and CD4⁺ to identify specific subpopulations. To determine the proportion of MAIT cells within the traditional CTL (CD8⁺) populations, CD3⁺ was used to identify T cells, which were then analyzed for CD4⁺ and CD8⁺ and both populations were then gated on V α 7.2⁺CD161⁺ and the proportion and number of MAIT cells was calculated (Online Resource 1). To ensure optical

validity for this study, Cytometer Setup & Tracking (CST: BD Biosciences, San Jose, CA, USA) beads were run prior to every experiment to ensure the median fluorescent intensity (MFI) of antigen positive low, intermediate, and high populations were comparable across multiple experiments over the course of the study. To minimize spectral overlap and the panel compensation, the panel was designed such that ubiquitous antigens with high MFI were placed in potentially problematic channels (i.e. CD45 was detected with antibodies conjugated to PerCPCy5.5). Full panel details and antibody concentrations can be found in Online Resource 2. Unlabeled and single color compensation controls were used for every experiment in addition to fluorescence minus one (FMO) controls for all antigens initially to test our ability to resolve populations; after which subsequent experiments employed a PE FMO for every experiment given that this channel was used to detect the major T cell subpopulation that was the subject of this investigation (i.e. invariant MAIT cells expressing the invariant T cell receptor variable alpha chain (V α 7.2). Cell viability was assessed using 7-AAD (7-amino-actinomycin D, Biolegend, San Diego, CA, USA) at time of analysis. All gating analysis was performed using FlowJo version 10.1r7 (Ashland, OR, USA). Circulating cell number was determined by multiplying the percentage of lymphocytes expressing the markers of interest with the hematology total lymphocyte count for each population.

Statistical Analysis

Changes in MAIT cell frequency were determined using a two-tailed paired samples T-test. Pearson product-moment correlations were determined between baseline MAIT cell numbers and the change with acute exercise for body composition and VO₂peak, and GXT duration. Statistical significance was set at $P < 0.05$. Data was reported as mean and standard deviation (SD). All analyses were performed using SPSS version 22 (SPSS, Inc., Chicago, IL,

USA). All figures were made using GraphPad Prism version 7 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Men in this study were lean and had moderate levels of aerobic fitness with an average exercise exposure of approximately 31 minutes (Table 1). All leukocyte populations significantly increased after maximal exercise (range 60.7 – 137%; all $P < 0.001$; Table 2) with the change in lymphocyte numbers being the most dramatic. Plasma volume changes with exercise were estimated at -20.5 (-5.1%, $P < 0.001$).

Insert Table 1 and 2 here.

The percentage of conventional T cells was altered following acute exercise. The percentage of $CD3^+$ cells significantly decreased ($P < 0.001$), $CD4^+$ T cell percentage also decreased but the change did not reach significance, while $CD8^+$ T cells increased significantly ($P < 0.001$; Fig. 1e). Absolute T cell counts significantly increased by 73 (33%), 48 (46%), and 102 (58%) for $CD3^+$, $CD4^+$, and $CD8^+$, respectively (all $P < 0.001$; Fig. 1f) following the GXT. The $CD4^+$ to $CD8^+$ ratio at baseline was 1.7 (0.8) and significantly decreased to 1.2 (0.6, $P < 0.001$) after exercise.

Insert Figure 1 here.

Slightly more than 3% of $CD3^+$ T cells were classified as $V\alpha 7.2^+CD161^+$ MAIT cells at baseline and this increased significantly following exercise ($P = 0.013$, Fig. 2c). Absolute MAIT cell counts were significantly elevated by 116 (90%) after exercise ($P < 0.001$, Fig. 2d).

Insert Figure 2 here.

Within MAIT cells, $CD8^+$ cells were the major subpopulation. The proportion of cells remained constant with exercise (Fig. 3c) while there was a 117 (114%) increase in absolute cell number ($P = 0.001$, Fig. 3d). MAIT cells that were $CD4^-CD8^-$ were the other significant

subpopulation and they followed the same pattern, with a 111 (107%) increase in cell number being the only change with exercise (P=0.002, Figure 3F).

Insert Figure 3 here.

As the majority (~87%) of MAIT cells were CD8⁺ and to address our secondary purpose, MAIT cells as a percentage of CTLs were examined before and after exercise. MAIT cells comprised slightly less than 10% of all CTLs and the proportion was unaltered with exercise (Fig. 4c).

Insert Figure 4 here.

To determine if aerobic fitness levels, exercise duration or body composition influenced the MAIT cells response to exercise, a correlation analysis was performed. No significant correlations were observed between MAIT cell counts and body composition, VO₂peak, or GXT duration at baseline or in the change with exercise (Table 3).

Insert Table 3 here.

DISCUSSION

The primary aim of this study was to assess changes in the V α 7.2⁺CD161⁺ MAIT cells and the respective CD8⁺ and CD4⁺CD8⁻ MAIT cell subpopulation response to acute, maximal aerobic exercise. Supporting our initial hypothesis, we observed for the first time that immediately following maximal exercise in healthy young men, there was a significant increase in absolute circulating MAIT cell counts. Additionally, MAIT cell proportions when expressed relative to total T cells significantly increased with exercise, suggesting a preferential mobilization into the blood (Figure 2). Neither the CD4⁺ (data not shown), CD8⁺ or CD4⁺CD8⁻ MAIT cell subpopulations accounted for the significant increase, suggesting small contributions from each population (Figure 3). Baseline and the change in MAIT cell populations with exercise were unaffected by GXT duration, fitness levels, or body composition (Table 3). Our secondary object was to assess MAIT cells within CTLs. We observed that V α 7.2⁺CD161⁺ MAIT cells comprised ~8% of CTLs and remained constant with acute exercise (Figure 4). Overall, these findings indicate that while exercise-induced lymphocytosis was the principal driving force behind the increased cell numbers, CD3⁺V α 7.2⁺CD161⁺ MAIT cells are preferentially mobilized within the T cell populations. While it remains unclear if increases in MAIT cell number result in improved function or tissue accumulation, it raises the possibility that exercise has the potential to boost mucosal immunity in patient populations presenting with low MAIT cell counts.

We report that resting MAIT cells make up ~3% of all T cells with the majority (87%) of MAIT cells also being CD8⁺, which is in agreement with ranges shown previously (Godfrey et al. 2015; Hinks et al. 2015; Magalhaes et al. 2015; Serriari et al. 2014) and appear to exceed numbers seen with asthma (~1%) (Hinks et al. 2015), type 2 diabetes (0.27%) or obesity (0.10%)

(Magalhaes et al. 2015). Following vigorous exercise lasting, on average, 31 minutes, circulating MAIT cell counts ($V\alpha 7.2^+CD161^+$, $V\alpha 7.2^+CD161^+CD8^+$, $V\alpha 7.2^+CD161^+CD4^-CD8^-$) increased more than 2-fold. As we are unaware of any studies examining MAIT cells and exercise, these innate-like T cells were compared to changes seen within classic T cell populations. In general, the percent change in MAIT cell counts (range: 111-116%) appear to exceed the increases within the $CD3^+$ (73%) and $CD3^+CD4^+$ (48%) cells and were similar to changes in $CD3^+CD8^+$ cell count (102%). Moreover, the improvement seen in the current study's classic T cell numbers strongly supports previous studies performing maximal exercise (Simpson et al. 2008) and exercise to exhaustion (Campbell et al. 2008).

The small but significant increase in MAIT cell percentage, expressed relative to total T cells, is an intriguing finding. Intense acute exercise induces greater $CD8^+$ cell proportion increases relative to total T cells, shown here (Figure 1E and F) and by others (Campbell et al. 2008; Gannon et al. 2001; Simpson et al. 2008). This greater rate of $CD8^+$ cell mobilization is readily identified by the change in the $CD4/CD8$ ratio. Most MAIT cells are also $CD8^+$, just under 90% in the current study (Figure 2F and G), but this percentage does not change after exercise. By gating for MAIT cells simultaneously along with the $CD8^+$ and $CD4^+$ T cell subpopulations, we are able to demonstrate the relative contribution of three distinct T cell populations with exercise-induced lymphocytosis. However, it is important to note that despite shifts towards greater MAIT cell proportion, the increased absolute counts in circulation are primarily due to the 137% increase in lymphocyte number, which is consistent with previous work using intense exercise (Campbell et al. 2008; Gannon et al. 2001; Nieman et al. 1992; Simpson et al. 2008).

Circulating MAIT cell deficiencies have been reported across multiple clinical populations including asthma (Hinks et al. 2015), irritable bowel syndrome, Crohn's Disease (Serriari et al. 2014), and with type 2 diabetes and obesity (Magalhaes et al. 2015). Interestingly, reductions in body mass and glycated hemoglobin (HbA1C) following bariatric surgery partially rescued MAIT cell abnormalities and attenuated inflammatory cytokine production (Magalhaes et al. 2015). Aerobic exercise training can also reduce total body and fat mass and HbA1C and improve insulin sensitivity (Maillard et al. 2016; Willis et al. 2012), which may partially alleviate the decrease in MAIT cell numbers. However, body mass and fat changes with exercise training are modest (2-3%), even in overweight and obese adults (Ohkawara et al. 2007; Willis et al. 2012). Alternatively, moderate intensity exercise training has been hypothesized to favor the anti-inflammatory T_h2 response but without complete suppression of T_h1 (Martin et al. 2009a). Downregulation of chronic inflammation may be another way that exercise affects cellular immunity (Martin et al. 2009b; Nieman 1994; Simpson et al. 2012; Walsh et al. 2011). While pro-inflammatory MAIT cells and their response to exercise training has not been studied, we hypothesize that regular exercise will alleviate chronic inflammation and reduce obesity and presents as a potential therapeutic option in individuals with low MAIT cell counts.

Although obesity reduces circulating MAIT cell numbers at rest and high intensity and long duration exercise enhance lymphocytosis (Gleeson and Bishop 2005), we failed to observe any relationship with baseline MAIT cells or the change with acute exercise. Previously, severe obesity (BMI >45) was associated with low MAIT cells (Carolan et al. 2015; Magalhaes et al. 2015) whereas the current study had participants of normal body mass and % fat. Total GXT duration was relatively constant, which likely precluded our ability to observe a relationship. VO₂peak not being associated with MAIT cell responses supports older work where T cells

responses occurred independent of fitness level (Kendall et al. 1990), although higher fitness levels attenuate T cell responses to submaximal exercise (Hong et al. 2005). Under the current conditions, GXT duration, cardiovascular fitness, and body composition did not influence the MAIT cell exercise response and may require values to be outside normal ranges before effects are seen.

Not accounting for MAIT cells within the CD8⁺ T cell population is a potential limitation within exercise immunology. As 10% of CD8⁺ T cell express MAIT cell markers (Cosgrove et al. 2013; Dusseaux et al. 2011; Fergusson et al. 2016; Walker et al. 2012), similar to observations from the current study, this indicates that CD8⁺Vα7.2⁺CD161⁺ MAIT cells are a substantial population with particular relevance given the preferential mobilization of CD8⁺ cells following exercise (Campbell et al. 2008; Gannon et al. 2001; Simpson et al. 2008). While CTLs and MAIT cells share similar cytotoxicity mechanisms (Kurioka et al. 2015), the traditional targets of these respective cells are viral and bacterial infections (Dusseaux et al. 2011; Gold et al. 2010), although recent evidence argues otherwise (Loh et al. 2016). However, failing to exclude MAIT cells when assessing classic CTL function likely underestimates normalized cytotoxic function. If viral infections activate and mobilize CTLs but also help prime MAIT cells for concurrent bacterial infections (Loh et al. 2016), this potentially enhanced immunity may benefit at risk populations during critical times. Boosting CD8⁺ (both CTLs and MAIT) cells with acute exercise to help maintain low viral and bacterial load during early infection is one possibility. However, the intensity of exercise required to illicit sufficient lymphocytosis must be carefully weighed (and tested in future studies) against the consequences of and guidelines for exercising during illness (Walsh et al. 2011).

This study was limited in that the MAIT cell response was determined in young males before and immediately after maximal exercise at two time points. Although these data are promising and are a necessary first step to determining the role of MAIT cells during exercise, several important questions remain unanswered. It remains unknown whether MAIT cells follow the biphasic response seen in other lymphocyte populations following exercise of sufficient intensity and duration (Gleeson and Bishop 2005). While cell number increases, does exercise alter MAIT cell function (i.e. cytokine production) or chemokine expression which aids in cellular trafficking to the mucosa? Is this response consistent across age, sex, and disease? Moreover, changes in circulating MAIT cell number and frequency may not reflect what is happening at the specific tissues. Previous studies have demonstrated decreased MAIT cells in the blood but conflicting findings in specific tissues (Hiejima et al. 2015; Serriari et al. 2014). Future studies may consider evaluating these parameters to allow for a more complete understanding of MAIT cell alterations within the context of exercise.

In conclusion, MAIT cell numbers are increased following maximal exercise and appear to be preferentially mobilized within the total T cell population. This mobilization occurs independent of exercise duration, fitness levels and body composition. The doubling of circulating MAIT cells with vigorous exercise suggests that this may be a means of offsetting MAIT cell deficiencies observed with some pathologies but this needs to be tested in future investigations. MAIT cells also make up a substantial proportion of CTLs, which may impact the interpretation of cytotoxicity assays within these cells.

FUNDING

This work was supported by the Collaborative Research Network from the Department of Industry, Innovation, and Science of Australia.

ACKNOWLEDGEMENTS

The authors would like to thank the volunteers for their involvement in this study and Ms. Shadney Que and Ms. Chantelle Blythe for their assistance in the laboratory.

REFERENCES

- Billerbeck E, Kang YH, Walker L, Lockstone H, Grafmueller S, Fleming V, Flint J, Willberg CB, Bengsch B, Seigel B, Ramamurthy N, Zitzmann N, Barnes EJ, Thevanayagam J, Bhagwanani A, Leslie A, Oo YH, Kollnberger S, Bowness P, Drognitz O, Adams DH, Blum HE, Thimme R, Klenerman P (2010) Analysis of CD161 expression on human CD8⁺ T cells defines a distinct functional subset with tissue-homing properties *Proc Natl Acad Sci U S A* 107:3006-3011 doi:10.1073/pnas.0914839107
- Bishop NC, Hayashida H, Clark M, Coombs C, Miller S, Stensel DJ (2014) Effect of acute and regular exercise on growth hormone secretagogue receptor-1a expression in human lymphocytes, T cell subpopulation and monocytes *Brain Behav Immun* 39:172-179 doi:10.1016/j.bbi.2013.09.017
- Campbell JP, Guy K, Cosgrove C, Florida-James GD, Simpson RJ (2008) Total lymphocyte CD8 expression is not a reliable marker of cytotoxic T-cell populations in human peripheral blood following an acute bout of high-intensity exercise *Brain Behav Immun* 22:375-380 doi:10.1016/j.bbi.2007.09.001
- Carolan E, Tobin LM, Mangan BA, Corrigan M, Gaoatswe G, Byrne G, Geoghegan J, Cody D, O'Connell J, Winter DC, Doherty DG, Lynch L, O'Shea D, Hogan AE (2015) Altered distribution and increased IL-17 production by mucosal-associated invariant T cells in adult and childhood obesity *J Immunol* 194:5775-5780 doi:10.4049/jimmunol.1402945
- Chen Z, Wang H, D'Souza C, Sun S, Kostenko L, Eckle SB, Meehan BS, Jackson DC, Strugnell RA, Cao H, Wang N, Fairlie DP, Liu L, Godfrey DI, Rossjohn J, McCluskey J, Corbett AJ (2017) Mucosal-associated invariant T-cell activation and accumulation after in vivo infection depends on microbial riboflavin synthesis and co-stimulatory signals *Mucosal Immunol* 10:58-68 doi:10.1038/mi.2016.39
- Cosgrove C, Ussher JE, Rauch A, Gartner K, Kurioka A, Huhn MH, Adelmann K, Kang YH, Fergusson JR, Simmonds P, Goulder P, Hansen TH, Fox J, Gunthard HF, Khanna N, Powrie F, Steel A, Gazzard B, Phillips RE, Frater J, Uhlig H, Klenerman P (2013) Early and nonreversible decrease of CD161⁺⁺ /MAIT cells in HIV infection *Blood* 121:951-961 doi:10.1182/blood-2012-06-436436
- Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration *J Appl Physiol* 37:247-248
- Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, Milder M, Le Bourhis L, Soudais C, Treiner E, Lantz O (2011) Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161^{hi} IL-17-secreting T cells *Blood* 117:1250-1259 doi:10.1182/blood-2010-08-303339
- Fergusson JR, Huhn MH, Swadling L, Walker LJ, Kurioka A, Llibre A, Bertoletti A, Hollander G, Newell EW, Davis MM, Sverremark-Ekstrom E, Powrie F, Capone S, Folgori A, Barnes E, Willberg CB, Ussher JE, Klenerman P (2016) CD161(int)CD8⁺ T cells: a

- novel population of highly functional, memory CD8⁺ T cells enriched within the gut
Mucosal Immunol 9:401-413 doi:10.1038/mi.2015.69
- Fernandez CS, Amarasena T, Kelleher AD, Rossjohn J, McCluskey J, Godfrey DI, Kent SJ (2015) MAIT cells are depleted early but retain functional cytokine expression in HIV infection Immunol Cell Biol 93:177-188 doi:10.1038/icb.2014.91
- Fyfe JJ, Bartlett JD, Hanson ED, Stepto NK, Bishop DJ (2016) Endurance Training Intensity Does Not Mediate Interference to Maximal Lower-Body Strength Gain during Short-Term Concurrent Training Front Physiol 7:487 doi:10.3389/fphys.2016.00487
- Gabriel H, Schwarz L, Born P, Kindermann W (1992) Differential mobilization of leucocyte and lymphocyte subpopulations into the circulation during endurance exercise Eur J Appl Physiol Occup Physiol 65:529-534
- Gannon GA, Rhind SG, Shek PN, Shephard RJ (2001) Differential cell adhesion molecule expression and lymphocyte mobilisation during prolonged aerobic exercise Eur J Appl Physiol 84:272-282 doi:10.1007/s004210000374
- Gherardin NA, Keller AN, Woolley RE, Le Nours J, Ritchie DS, Neeson PJ, Birkinshaw RW, Eckle SB, Waddington JN, Liu L, Fairlie DP, Uldrich AP, Pellicci DG, McCluskey J, Godfrey DI, Rossjohn J (2016) Diversity of T Cells Restricted by the MHC Class I-Related Molecule MR1 Facilitates Differential Antigen Recognition Immunity 44:32-45 doi:10.1016/j.immuni.2015.12.005
- Gleeson M, Bishop NC (2005) The T cell and NK cell immune response to exercise Ann Transplant 10:43-48
- Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB (2015) The burgeoning family of unconventional T cells Nat Immunol 16:1114-1123 doi:10.1038/ni.3298
- Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J, Winata E, Swarbrick GM, Chua WJ, Yu YY, Lantz O, Cook MS, Null MD, Jacoby DB, Harriff MJ, Lewinsohn DA, Hansen TH, Lewinsohn DM (2010) Human mucosal associated invariant T cells detect bacterially infected cells PLoS Biol 8:e1000407 doi:10.1371/journal.pbio.1000407
- Gold MC, Eid T, Smyk-Pearson S, Eberling Y, Swarbrick GM, Langley SM, Streeter PR, Lewinsohn DA, Lewinsohn DM (2013) Human thymic MR1-restricted MAIT cells are innate pathogen-reactive effectors that adapt following thymic egress Mucosal Immunol 6:35-44 doi:10.1038/mi.2012.45
- Gold MC, McLaren JE, Reistetter JA, Smyk-Pearson S, Ladell K, Swarbrick GM, Yu YY, Hansen TH, Lund O, Nielsen M, Gerritsen B, Kesmir C, Miles JJ, Lewinsohn DA, Price DA, Lewinsohn DM (2014) MR1-restricted MAIT cells display ligand discrimination and pathogen selectivity through distinct T cell receptor usage J Exp Med 211:1601-1610 doi:10.1084/jem.20140507

- Gold MC, Napier RJ, Lewinsohn DM (2015) MR1-restricted mucosal associated invariant T (MAIT) cells in the immune response to Mycobacterium tuberculosis Immunol Rev 264:154-166 doi:10.1111/imr.12271
- Hansen JB, Wilsgard L, Osterud B (1991) Biphasic changes in leukocytes induced by strenuous exercise Eur J Appl Physiol Occup Physiol 62:157-161
- Hiejima E, Kawai T, Nakase H, Tsuruyama T, Morimoto T, Yasumi T, Taga T, Kanegane H, Hori M, Ohmori K, Higuchi T, Matsuura M, Yoshino T, Ikeuchi H, Kawada K, Sakai Y, Kitazume MT, Hisamatsu T, Chiba T, Nishikomori R, Heike T (2015) Reduced Numbers and Proapoptotic Features of Mucosal-associated Invariant T Cells as a Characteristic Finding in Patients with Inflammatory Bowel Disease Inflamm Bowel Dis 21:1529-1540 doi:10.1097/MIB.0000000000000397
- Hinks TS, Zhou X, Staples KJ, Dimitrov BD, Manta A, Petrossian T, Lum PY, Smith CG, Ward JA, Howarth PH, Walls AF, Gadola SD, Djukanovic R (2015) Innate and adaptive T cells in asthmatic patients: Relationship to severity and disease mechanisms J Allergy Clin Immunol 136:323-333 doi:10.1016/j.jaci.2015.01.014
- Hong S, Johnson TA, Farag NH, Guy HJ, Matthews SC, Ziegler MG, Mills PJ (2005) Attenuation of T-lymphocyte demargination and adhesion molecule expression in response to moderate exercise in physically fit individuals J Appl Physiol (1985) 98:1057-1063 doi:10.1152/jappphysiol.00233.2004
- Kendall A, Hoffman-Goetz L, Houston M, MacNeil B, Arumugam Y (1990) Exercise and blood lymphocyte subset responses: intensity, duration, and subject fitness effects J Appl Physiol (1985) 69:251-260
- Koay HF, Gherardin NA, Enders A, Loh L, Mackay LK, Almeida CF, Russ BE, Nold-Petry CA, Nold MF, Bedoui S, Chen Z, Corbett AJ, Eckle SB, Meehan B, d'Udekem Y, Konstantinov IE, Lappas M, Liu L, Goodnow CC, Fairlie DP, Rossjohn J, Chong MM, Kedzierska K, Berzins SP, Belz GT, McCluskey J, Uldrich AP, Godfrey DI, Pellicci DG (2016) A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage Nat Immunol 17:1300-1311 doi:10.1038/ni.3565
- Kurioka A, Ussher JE, Cosgrove C, Clough C, Fergusson JR, Smith K, Kang YH, Walker LJ, Hansen TH, Willberg CB, Klenerman P (2015) MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets Mucosal Immunol 8:429-440 doi:10.1038/mi.2014.81
- Loh L, Wang Z, Sant S, Koutsakos M, Jegaskanda S, Corbett AJ, Liu L, Fairlie DP, Crowe J, Rossjohn J, Xu J, Doherty PC, McCluskey J, Kedzierska K (2016) Human mucosal-associated invariant T cells contribute to antiviral influenza immunity via IL-18-dependent activation Proc Natl Acad Sci U S A 113:10133-10138 doi:10.1073/pnas.1610750113
- Magalhaes I, Pingris K, Poitou C, Bessoles S, Venteclef N, Kiaf B, Beaudoin L, Da Silva J, Allatif O, Rossjohn J, Kjer-Nielsen L, McCluskey J, Ledoux S, Genser L, Torcivia A,

- Soudais C, Lantz O, Boitard C, Aron-Wisnewsky J, Larger E, Clement K, Lehuen A (2015) Mucosal-associated invariant T cell alterations in obese and type 2 diabetic patients *J Clin Invest* 125:1752-1762 doi:10.1172/JCI78941
- Maillard F, Rousset S, Pereira B, Traore A, de Pradel Del Amaze P, Boirie Y, Duclos M, Boisseau N (2016) High-intensity interval training reduces abdominal fat mass in postmenopausal women with type 2 diabetes *Diabetes Metab* 42:433-441 doi:10.1016/j.diabet.2016.07.031
- Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, Premel V, Devys A, Moura IC, Tilloy F, Cherif S, Vera G, Latour S, Soudais C, Lantz O (2009a) Stepwise development of MAIT cells in mouse and human *PLoS Biol* 7:e54 doi:10.1371/journal.pbio.1000054
- Martin SA, Pence BD, Woods JA (2009b) Exercise and respiratory tract viral infections *Exerc Sport Sci Rev* 37:157-164 doi:10.1097/JES.0b013e3181b7b57b
- Nieman DC (1994) Exercise, upper respiratory tract infection, and the immune system *Med Sci Sports Exerc* 26:128-139
- Nieman DC, Henson DA, Johnson R, Lebeck L, Davis JM, Nehlsen-Cannarella SL (1992) Effects of brief, heavy exertion on circulating lymphocyte subpopulations and proliferative response *Med Sci Sports Exerc* 24:1339-1345
- Ohkawara K, Tanaka S, Miyachi M, Ishikawa-Takata K, Tabata I (2007) A dose-response relation between aerobic exercise and visceral fat reduction: systematic review of clinical trials *Int J Obes (Lond)* 31:1786-1797 doi:10.1038/sj.ijo.0803683
- Rossiter HB, Kowalchuk JM, Whipp BJ (2006) A test to establish maximum O₂ uptake despite no plateau in the O₂ uptake response to ramp incremental exercise *J Appl Physiol* (1985) 100:764-770 doi:10.1152/jappphysiol.00932.2005
- Serriari NE, Eoche M, Lamotte L, Lion J, Fumery M, Marcelo P, Chatelain D, Barre A, Nguyen-Khac E, Lantz O, Dupas JL, Treiner E (2014) Innate mucosal-associated invariant T (MAIT) cells are activated in inflammatory bowel diseases *Clin Exp Immunol* 176:266-274 doi:10.1111/cei.12277
- Simpson RJ, Cosgrove C, Ingram LA, Florida-James GD, Whyte GP, Pircher H, Guy K (2008) Senescent T-lymphocytes are mobilised into the peripheral blood compartment in young and older humans after exhaustive exercise *Brain Behav Immun* 22:544-551 doi:10.1016/j.bbi.2007.11.002
- Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC, Kunz H (2012) Exercise and the aging immune system *Ageing Res Rev* 11:404-420 doi:10.1016/j.arr.2012.03.003
- Takahashi T, Dejbakhsh-Jones S, Strober S (2006) Expression of CD161 (NKR-P1A) defines subsets of human CD4 and CD8 T cells with different functional activities *J Immunol* 176:211-216

- Tilloy F, Treiner E, Park SH, Garcia C, Lemonnier F, de la Salle H, Bendelac A, Bonneville M, Lantz O (1999) An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals *J Exp Med* 189:1907-1921
- Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, Affaticati P, Gilfillan S, Lantz O (2003) Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1 *Nature* 422:164-169 doi:10.1038/nature01433
- Ussher JE, Klenerman P, Willberg CB (2014) Mucosal-associated invariant T-cells: new players in anti-bacterial immunity *Front Immunol* 5:450 doi:10.3389/fimmu.2014.00450
- Walker LJ, Kang YH, Smith MO, Tharmalingham H, Ramamurthy N, Fleming VM, Sahgal N, Leslie A, Oo Y, Geremia A, Scriba TJ, Hanekom WA, Lauer GM, Lantz O, Adams DH, Powrie F, Barnes E, Klenerman P (2012) Human MAIT and CD8alphaalpha cells develop from a pool of type-17 precommitted CD8+ T cells *Blood* 119:422-433 doi:10.1182/blood-2011-05-353789
- Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A, Simon P (2011) Position statement. Part one: Immune function and exercise *Exerc Immunol Rev* 17:6-63
- Willis LH, Slentz CA, Bateman LA, Shields AT, Piner LW, Bales CW, Houmard JA, Kraus WE (2012) Effects of aerobic and/or resistance training on body mass and fat mass in overweight or obese adults *J Appl Physiol* (1985) 113:1831-1837 doi:10.1152/jappphysiol.01370.2011
- Zhang N, Bevan MJ (2011) CD8(+) T cells: foot soldiers of the immune system *Immunity* 35:161-168 doi:10.1016/j.immuni.2011.07.010

TABLES

Table 1. Participant characteristics.

Age (y)	28 (5)
Height (cm)	180.6 (6.4)
Mass (kg)	81.1 (14.1)
Fat mass (kg)	15.0 (7.0)
Fat free mass (kg)	61.7 (7.7)
Body fat (%)	17.8 (6.0)
VO ₂ peak (ml/kg/min)	44.4 (10.2)
GXT duration (min)	31.3 (6.1)
Mean (SD)	
GXT = graded exercise test	
VO ₂ peak = peak oxygen uptake	
Fat free mass = Total mass – Fat mass – Bone mineral content	

Table 2. Whole blood parameters before and after maximal exercise.

	Baseline	Post exercise	% Change	P value
Red blood cells (x10 ⁶ cells/μl)	4.6 (0.3)	5.2 (0.3)	13.2	<0.001
White blood cells (x10 ³ cells/μl)	5.3 (1.4)	9.9 (2.3)	86.8	<0.001
Lymphocytes (x10 ³ cells/μl)	1.9 (0.6)	4.5 (1.5)	136.8	<0.001
Mixed cells (x10 ³ cells/μl)	0.5 (0.2)	1.0 (0.3)	100.0	<0.001
Neutrophils (x10 ³ cells/μl)	2.8 (0.9)	4.5 (1.1)	60.7	<0.001
Hemoglobin (g/dL)	14.4 (0.8)	16.2 (0.9)	12.5	<0.001
Hematocrit (%)	41.2 (2.4)	47.3 (2.6)	14.8	<0.001
Mean (SD)				

Table 3. Pearsons correlation coefficients for MAIT cell numbers at baseline and the change with exercise with body composition, VO₂peak, and exercise duration.

	Fat mass (kg)	Body fat (%)	Lean mass (kg)	VO ₂ peak (ml/kg/min)	GXT duration (min)
Baseline					
Vα7.2 ⁺ CD161 ⁺	-0.02	-0.01	-0.02	0.06	-0.12
Vα7.2 ⁺ CD161 ⁺ CD8 ⁺	-0.04	-0.03	-0.05	0.07	-0.15
Change					
Vα7.2 ⁺ CD161 ⁺	0.29	0.31	0.07	0.01	-0.00
Vα7.2 ⁺ CD161 ⁺ CD8 ⁺	0.31	0.32	0.09	-0.01	-0.13
GXT = graded exercise test; VO ₂ peak = peak oxygen uptake					

Online Material 2. Flow Cytometer Optical Layout and Antibody Quantities.

Laser	Detector/PMT	Antigen	Fluorochrome	Amount	Clone
Blue (488nm)	B585/42	V α 7.2	PE	0.1 μ g	3C10
	B670	CD45	PerCP Cy5.5	0.05 μ g	HI30
	B780/60	CD8	PE-Cy7	0.2 μ g	HIT8a
Red (633nm)	R780/60	CD3	APC-Cy7	0.2 μ g	UCHT1
Violet (405nm)	V450/50	CD161	Brilliant Violet 421	0.2 μ g	HP-3G10
	V525/50	CD4	Brilliant Violet 500	-	RPA-T4

FIGURE CAPTIONS

Fig. 1 The changes in classical T cells and T cell subpopulations with maximal aerobic exercise. Representative FACS plots of CD3⁺ T cells gated on lymphocytes **A)** before and **B)** after exercise and the CD4⁺ and CD8⁺ subpopulations gated on CD3 **C)** before and **D)** after exercise. The plot closest to the mean value at baseline was used for both timepoints. **E)** The changes in T cell populations expressed as a percentage of their parent population and **F)** the increased in absolute number of T cell after exercise. Absolute cell number was determined by multiplying the cell proportions as a percentage of lymphocytes and multiplying by the total lymphocyte count. Data expressed as mean (SD)

Fig. 2 V α 7.2⁺CD161⁺ MAIT increase in frequency and number with maximal aerobic exercise. Representative FACS plots of MAIT cells gated on CD3⁺ T cells **A)** before and **B)** after exercise. The plot closest to the mean value at baseline was used for both timepoints. **C)** V α 7.2⁺CD161⁺ MAIT cell as a percentage of total T cells and **D)** absolute MAIT cell counts both increased following exercise. Data expressed as mean (SD)

Fig. 3 MAIT cell subpopulations (CD8⁺ and double negative) do not change following maximal aerobic exercise. Representative FACS plots of the proportion of MAIT (V α 7.2⁺CD161⁺) cell subpopulations expressing CD4 and CD8 **A)** at baseline and **B)** after exercise. The plot closest to the mean value at baseline was used for both timepoints. **C)** The percentage of MAIT cells that express only CD8 and **D)** the absolute cell number and the **E)** the percentage of MAIT cells that did not express CD4 or CD8 and **F)** the absolute cell number remained constant following exercise. Data are expressed as mean (SD)

Fig. 4 Representative FACS plots of the proportion of CTLs that express V α 7.2⁺CD161⁺ MAIT cell markers **A)** at baseline and **B)** after exercise. The plot closest to the mean value at baseline was used for both timepoints. **C)** CTLs were identified as CD3⁺CD8⁺ and then gated on V α 7.2⁺CD161⁺. The percentage of CTLs that appear as MAIT cells was unchanged with exercise. Data expressed as mean (SD)

SUPPLEMENTAL FIGURE CAPTION

Supplemental Fig. 1 Immunofluorescence gating strategy for the identification of the proportion of (relative to the parent population) and the absolute numbers of MAIT cells with acute exercise. **A)** Gating of single PBMCs, **B)** the inclusion of CD45⁺ cells, **C)** identifying lymphocytes, and **D)** gating for T (CD3⁺) and the **E)** CD4⁺ and CD8⁺ T cell subpopulations. Subsequently, cells that express V α 7.2 and CD161 were determined from **F)** cytotoxic T lymphocytes CDLs (CD8⁺) and **G)** CD4. **H)** From the total T cell population (panel D), V α 7.2⁺CD161⁺ MAIT cells were identified and the **G)** MAIT cell subpopulations of CD4⁺ and CD8⁺.